Amer. Malac. Bull. **27**: 105-111 (2009)

The use of developmental sequences for assessing evolutionary change in gastropods*

Jennifer Smirthwaite, Simon D. Rundle, and John I. Spicer

School of Biological Sciences, University of Plymouth, Plymouth PL4 8AA, U.K.

Corresponding author: srundle@plymouth.ac.uk

Abstract: First introduced by Ernst Haeckel in the nineteenth century, the use of developmental sequences has recently seen a renaissance as part of the study of the evolutionary biology of embryos; here we review briefly the literature describing gastropod developmental sequences, appraising the extent to which it has contributed to this renaissance. Gastropods have figured extensively in studies of early development with cell lineage analysis available for numerous taxa. Phylogenetic comparisons of these data reveal strong evolutionary signals, particularly in relation to early cell divisions. In contrast, although the description of post cell division developmental stages, including functional elements of development, in gastropods is extensive, interspecific comparisons are rare and tend to focus instead on developmental mode. However, a recent comparison of the sequence of functional and morphological events in a clade of basommatophoran snails demonstrated several alterations in the timing of developmental events (*i.e.*, heterochronies) across the phylogeny. Many gastropod groups may offer the potential to carry out similar investigations of the evolutionary importance of sequence heterochrony and to try to unravel the mechanistic basis for such patterns in developmental sequences.

Key words: Pulmonata, Basommatophora, embryo, development, heterochrony

"*....Haeckel can be seen as the father of a sequence-based phylogenetic embryology*" (Richardson and Keuck 2002: 495).

DEVELOPMENTAL SEQUENCES IN BIOLOGY

Haeckel is perhaps best known for his Biogenetic Law, which, in its simplest interpretation, proposed that ontogeny is a brief and rapid re-run of phylogeny, evolution occurring by terminal addition (Haeckel 1866). Much has been made of the rescinding of this law, and Haeckel's reputation and work has to some extent been damaged by these discussions (Garstang 1922, deBeer 1958, Gould 1977, Richardson 1995, 1998, Richardson and Jeffery 2002, Richardson and Keuck 2002). However, it is frequently overlooked that, in the process of formulating his now discredited law, Haeckel made other valuable contributions to the field of evolutionary biology (Richardson and Keuck 2002). First, he flagged the importance of variation in embryonic development between species, the same variation that underpins much of the current emphasis in "Evo-Devo" research (Raff 2000, Arthur 2002). Second, he was first to champion the use of developmental sequences in cross-species comparisons (Richardson and Keuck 2002): his approach of using letters of the alphabet to represent the succession of developmental stages that could be lost or replaced through evolutionary time provided a significant advance on other approaches that focused on the relative rates of development of different traits (*e.g.*, as emphasized by workers such as Wilhelm His) and is very similar to approaches currently being advocated for analyzing changes in developmental sequence between descendent and ancestral species (*i.e.*, heterochronies—see below).

Related to the use of developmental sequences, another of Haeckel's major contributions to biology was the coining of the term *heterochrony*, which he used to describe "anomalies" to his biogenetic law (Richardson and Keuck 2002). Such anomalies were alterations to developmental sequences across species, whereby developmental events appeared either later or earlier in ontogeny (Haeckel also flagged heterotopes, which were alterations in the position of developmental events within the embryo). While Haeckel viewed heterochronies as exceptions to his biogenic law, they appear to have assumed major importance in the investigation of evolution and have even been suggested by some to be one of the key drivers of evolutionary change (*e.g.*, Gould 1977, but see also Raff 1996). Much of this emphasis of heterochrony as a major evolutionary shaping force has centered around shifts in the developmental timing of morphological traits and, in particular, body size (as a proxy for developmental time) in relation to reproductive maturation. This so-called global heterochrony shifted the emphasis from sequences to one concerned mostly with size and shape (*i.e.*, towards an allometric approach).

* From the symposium "Molluscs as models in evolutionary biology: from local speciation to global radiation" presented at the World Congress of Malacology, held from 15 to 20 July 2007 in Antwerp, Belgium.

It is only comparatively recently that those interested in heterochrony have re-established the Haeckelian focus on using developmental sequences and the potential importance of comparing these sequences across taxa in order to elucidate patterns in the evolution of development (*e.g.*, Smith 2001, 2003, Bininda-Emonds *et al.* 2002). This "new" emphasis has seen the development of sophisticated analytical procedures that allow the formal comparisons of changes in developmental sequence across species within an explicit phylogenetic context (sequence heterochronies) (Mabee and Trendler 1996, Smith 1996, 1997, 2001, Richardson *et al.* 2001, Bininda-Emonds *et al.* 2002, Jeffery *et al.* 2002a, 2002b, 2005, Schulmeister and Wheeler 2004). Given the great wealth of information that exists on gastropod development, stretching back over one hundred years, coupled with a voluminous recent literature marrying molecular techniques with classical embryology, the question can be posed as to what extent has the study of gastropod development figured in this renaissance? Here we address this question by reviewing briefly work that has aimed to compare developmental sequences in gastropods. We demonstrate that work on gastropods has been at the forefront of investigations assessing the evolutionary importance of very early developmental stages but that, paradoxically, despite a voluminous literature documenting patterns for individual species, cross-species comparisons of events later in the developmental sequence appear to be rare. Finally, we present the findings of a recent study that begins, in a small way, to redress this imbalance.

Developmental sequences in gastropods

Cell lineage analysis

There is a long and distinguished history of studying cell lineages in gastropods, initiated by workers such as Blochmann (1882), Conklin (1897), and Delsman (1914) [see Raven (1958) and Fretter and Graham (1962) for summaries of this early work and Lindberg and Guralnick (2003) for a list of papers]. However, it was not until Hyman (1951) that the information from these pioneering studies alongside that on cell lineages for other spiralian taxa were used to propose a link between development and evolution; this reluctance to link ontogeny with phylogeny was almost certainly a reaction to the controversy surrounding recapitulation (Lindberg and Guralnick 2003).

In the past couple of decades, with the advent of sophisticated approaches for exploring phylogenetic relationships, gastropods have again been at the center of research linking early cell-cleavage patterns with evolution although it should be noted that many of these analyses draw on data from the studies at the turn of the nineteenth century (Freeman and Lundelius 1992, van den Biggelar 1993, van den Biggelar and Haszprunar 1996, Ponder and Lindberg 1997). In particular, there has been a focus on the timing of the formation of the 4d mesentoblast (the precursor of the mesoderm), with the relation of this timing to the cleavage of other cells being linked to the evolution of the major gastropod groups (van den Biggelar and Haszpruner 1996, Lindberg and Guralnick 2003). The main observation is that the onset of the 3d macromere division, which leads to the formation of the 4d mesentoblast, is accelerated through evolutionary time. Hence, in more derived gastropod groups such as the caenogastropods and heterobranchs, it occurs at the 24-cell stage compared with at the 63-cell stage in the stem gastropod taxa (*e.g.*, Patellogastropoda and Vestigastropoda). In effect, these studies demonstrate a heterochrony in the sequence of very early development, with a shift in the timing of developmental events between ancestral and descendent taxa.

A more recent analysis on more extensive cell-lineage data for more taxa (Lindberg and Guralinick 2003) confirmed that there was congruence between phylogenetic trees derived using cell lineages and those using morphological and molecular approaches. This study also identified a long branch within the cell-lineage phylogeny, indicating a large number of developmental event changes, between the Patellogastropoda/Vestigastropoda and Neritopsina/Apogastropoda clades. This evolutionary change again indicated an acceleration in development, a shortening of the trochophore stage, and an accompanying lengthening of the veliger stage. It was proposed that this shift towards the earlier development of a longer planktotrophic stage may have been a response to increased levels of primary production in the oceans during the Silurian period. In effect, this is a heterochronous change in the timing of developmental stage, and it is clear that the investigation of cell lineages in relation to gastropod evolution has led us full circle in terms of the important link between ontogeny and phylogeny.

Sequences in later developmental events

The evidence for an evolutionary role for development from extensive phylogenetic analysis of very early gastropod developmental events has not been extended to any great degree to the later stages of development. This is somewhat surprising, given the extent to which the developmental events of many gastropod species have been described in detail [for example see Raven (1958) and Fretter and Graham (1962)]. Indeed, the only substantial phylogenetic analysis on gastropod development has focused on an assessment of the evolution of developmental mode rather than developmental sequences *per se*. Collin (2004) mapped the developmental mode (of 72 calyptraeid gastropods) onto a phylogeny and found that there was no evidence that phylogenetic effects had constrained the evolution of this trait; species with planktotrophic, lecithotrophic, or direct development with

nurse eggs all had the potential to evolve a different developmental mode.

Other studies that focus more on developmental sequences are either qualitative or restricted in their comparative element. Page (1994), for example, provided an interesting qualitative comparison of the occurrence of eight developmental structures in opisthobranchs and prosobranchs. She concluded that young planktonic opisthobranch larvae represented a good approximation of an ancestral gastropod larva by not expressing many of the structures of the definitive body found early in prosobranch development (*i.e.*, at the veliger stage) until late in the larval phase. Gibson (2003), in contrast, found that one group of opisthobranchs, the Notospidea, possessed adult characters (notum differentiation, adult shell growth, lack of operculum) during the early larval stage. Collin and Wise (1997) included observations of early cell development in their investigation of development in the pyramidellid *Odostomia columbiana* Dall and Bartsch, 1907 and concluded that larvae with unequal cleavage and early development of eyes and tentacles might represent the common ancestors of pyramellids and opisthobranchs. At the same time, gastropods have been used by workers taking a functional approach to embryonic development, including studies of embryonic ionic balance (*e.g.*, Taylor 1977), calcification (*e.g.*, Bielefeld and Becker 1991), respiration (*e.g.*, Baldwin 1935), and muscle and nerve development (*e.g.*, Croll and Voronezhskaya 1996, Page 1998, Yamanaka *et al.* 2000).

Clearly, there is a lot of information on the later developmental stages of gastropod species both in terms of morphological and functional traits, including some evidence for differences in developmental sequences of these traits between species. More extensive, formal analysis of these data could shed much light on the role that developmental sequences have played in gastropod evolution; indeed, gastropods might provide particularly good models for integrated approaches that focus on functional as well as morphological aspects of development. In the next section, we describe a study that takes such a formal and integrative approach.

Case study: A quantitative comparison of developmental sequences in basommatophoran gastropods

So far, we have seen that there is an imbalance between cell lineage and later developmental characters when it comes to comparative approaches with developmental sequences in gastropods, with a lack of studies focusing on later development. In fact, the bulk of studies explicitly testing for differences in developmental sequences across phylogenies have been for mammals. In these examples, there is clear evidence for heterochrony in terms of altered *sequences* of developmental events between ancestors and descendents. There are, for example, differences in the timing of the central nervous system and the craniofacial apparatus between eutherians and marsupials (Smith 1997, Nunn and Smith 1998). Jeffery *et al.* (2002a) also demonstrated that, within the amniotes, mammals were characterized by delayed development of the eyes and that there were several heterochronies involving shifts in cardiac events relative to non-cardiac events. However, a more recent study suggested that the role of sequence heterochrony in mammals may not be that extensive; Bininda-Emonds *et al.* (2004) hypothesized that a lack of heterochronies in mammals might be expected due to the time during which the organo-genetic period occurs being spent in the protective environment of the amniotic egg. In many invertebrate groups, however, including many gastropods, embryonic development is external and, potentially, more open to evolutionary processes. Hence, we might predict that alterations to the developmental sequence might be more pronounced in such instances.

A recent study by Smirthwaite *et al.* (2007) took the first step towards exploring heterochrony in invertebrates, by comparing developmental sequences in three clades of basommatophoran snails. This work involved observing the precise timings of "traditional" morphological stages and functional/physiological traits in 12 species, mapping these events onto developmental time lines and then making a formal comparison of the relative timing of these events among species.

The morphological stages used were those that have been described previously by workers such as Raven (1958) and Cummin (1972) and included the trochophore, veliger, and hippo stages (Figs. 1A-C). It is important to note that it was the *onset* of these stages that were used as events. The physiological and functional events typically used occurred after these morphological stages (but see below) and included the formation of eyespots, heart, and radula, the onset of body flexing and contraction of mantle muscle, the time when the animal migrated from the egg capsule and, finally, the egg mass (Figs. 1-2).

Once event timings had been determined, timelines were constructed for each species, with that for *Lymnaea stagnalis* (Linnaeus, 1758) used as the "standard" (Fig. 2) and event-pair cracking (PARSIMOV approach—Jeffery *et al.* 2005) was used to test formally for heterochronies. In essence, this technique involves comparing the timing of each pair of events by classifying each event depending on whether it occurred earlier, simultaneously, or later than each other event in all other species. These scores are then mapped onto the phylogeny for the group.

This analysis provided formal support for several heterochronies, some of which are illustrated in Fig. 2. The lines on the first panel of this figure (*i.e.*, Fig. 2A) illustrate three traits (hippo, eyespot formation, and mantle muscle control) that do not change their timing relative to one another

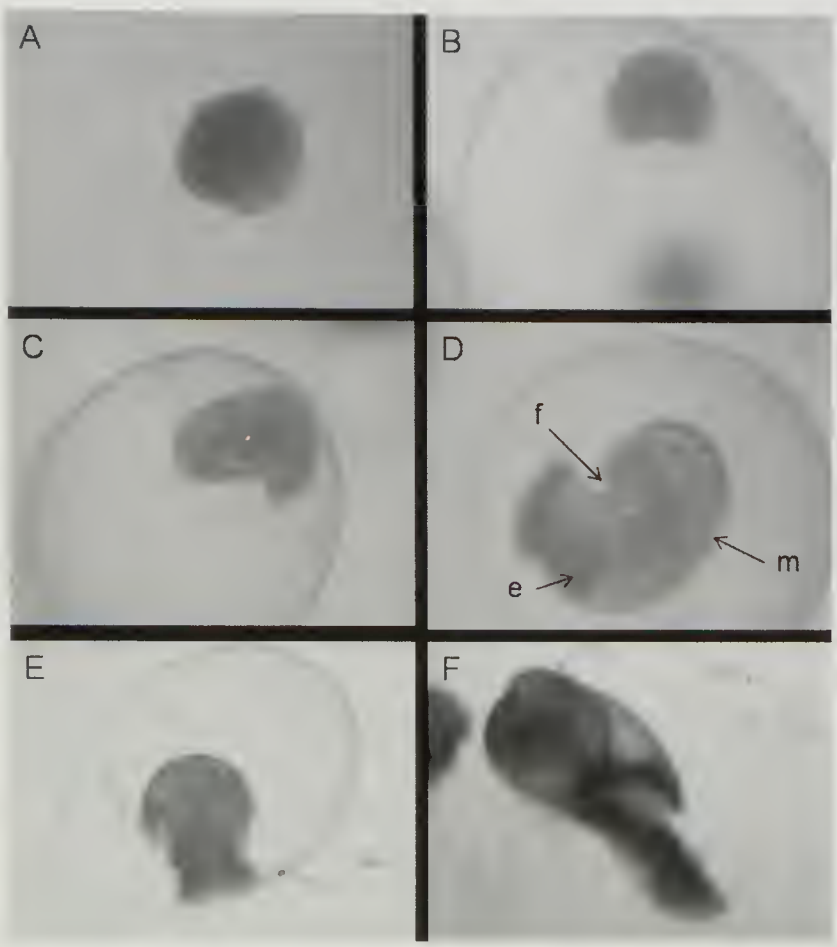

Figure 1. Developmental stages of *Lymnaea stagnalis* embryos: A, trochophore; B, veliger; C, early hippo; D, free-swimming hippo; E, crawling hippo; F, juvenile snail migrating from egg mass. Arrows and letter for free-swimming hippo indicate the location of the eye (e), body flexing (f), and mantle muscle contraction (m).

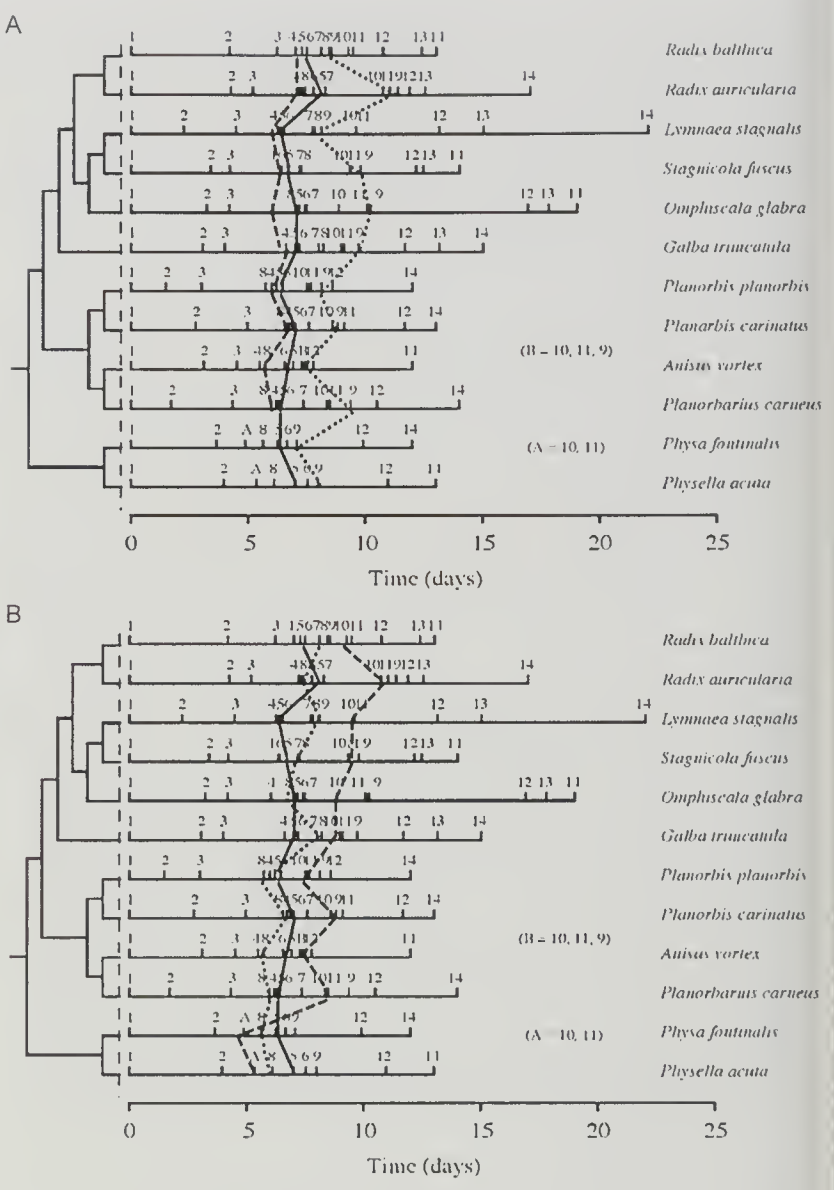

Figure 2. Sequences of events mapped on a time line for twelve species of basommatophoran snail; the three main clades represent (from top to bottom) the Lymnaeidae, Planorbidae, and Physidae. Event labeling (note: event timing was measured as the starting point for that event): 1, laying; 2, trochophore; 3, veliger; 4, hippo; 5, eyespot formed; 6, heart beat; 7, free swimming; 8, body flexing; 9, mantle muscle contraction; 10, attachment to egg; 11, crawling; 12, radula; 13, emergence from egg capsule; 14, migration from the egg mass. A, illustrates three non-heterochronous events, *i.e.*, which do not change their relative sequence across species: hippo, dashed line; eyespot formation, solid line; mantle muscle control, dotted line. B, illustrates sequence heterochronies among three events: in the two *Physa* species, attachment to the egg capsule (solid line) occurs earlier in development than body flexing (dotted line) and eyespot formation (dashed line) compared with in other species; body flexing (dotted line) occurs earlier in the Physidae, Planorbidae, and two species of Lymnaeidae (*Radix balthica* Linnaeus, 1758 and *Omphiscola glabra*) than in the other species within Lymnaeidae.

across species; hence, these traits are non-heterochronous. In contrast, the second panel (Fig. 2B) illustrates three traits that are heterochronous. One of these heterochronies involves the early occurrence of the embryo attaching to the egg capsule wall in relation to eyespot formation and body flexing in the two species of *Physa* Draparnaud, 1801 compared with all other species. Eyespot formation and body flexing were also heterochronous; within the Planorbidae and the Physidae, body flexing occurred before the eye was formed, whereas only two [*Radix auricularia* (Linnaeus, 1758) and *Omphiscola glabra* (O. F. Müller, 1774)] of the six species in the family Lymnaeidae showed this timing pattern. Hence, it appears that, within this clade of gastropods, heterochronies are associated with speciation events and larger taxonomic divergences at the family level. The number of events changing their position was also similar in relative terms to those identified for mammals (Bininda-Emonds *et al.* 2004).

Where can the investigation of developmental sequences in gastropods take us?

The study by Smirthwaite *et al.* (2007) provides evidence that sequence heterochrony occurs in a clade of gastropods, which suggests that, potentially, heterochrony make have played a part in the evolution of this group. However, we must be wary of assuming that, because we have demonstrated

heterochrony as essentially a *pattern* of evolutionary change, that the evolutionary mechanism must also involve heterochrony; there is no necessity for pattern and mechanism of evolutionary change both to be heterochronous (Spicer and Rundle 2007). Indeed, we would suggest that one of the big challenges for research on developmental sequences will be to establish whether there is a link between heterochronic process and heterochrony as a pattern (Spicer and Rundle 2007). Clearly, this approach will need to be guided by comparative studies such as that by Smirthwaite *et al.* (2007) that flag functional heterochronies between extant species, allowing investigations such as whether alterations to developmental sequences between species can be replicated within species. Such intraspecific changes in developmental sequence have been termed heterokairies (Spicer and Burggren 2003, Spicer and Rundle 2006, 2007) and their investigation should promote research on developmental sequences.

So might gastropods be a good model for future studies on the role of developmental sequences in evolution? We feel that the answer to this question is yes, for several reasons. First, the embryonic development of many gastropods is external and visible, which means that observations of early development and manipulations of the developmental environment are tractable. Second, phylogenies have been constructed for several groups and the relatedness of the major gastropod clades has also been formulated, allowing the explicit test of patterns in sequences within an evolutionary framework. Finally, the extensive information on cell lineages within the gastropods has meant that they have been one of the groups at the forefront of the development of techniques for generating cell-fate maps such as fluorescent stains used with confocal microscopy. Such techniques have already allowed some workers to trace developmental pathways from cell cleavage through to structures such as nerves, muscles, the mantle, and cilia within gastropod species (Render 1997, Hejnol *et al.* 2007, Wanninger *et al.* 2008). The emphasis so far has been in elucidating how major taxonomic divergences may be linked with different developmental pathways, yet it might be possible that species-level divergence might also be driven by small-scale variation in induction patterns during early cell divisions. Such variation might also be linked to later differences in the developmental sequence. Clearly, this is a highly speculative hypothesis but perhaps one that deserves consideration. At the same time, the ability to measure gene expression and up-regulation in gastropod embryos (Lartillot *et al.* 2002, Hinman *et al.* 2003) will also enhance our understanding of the genetic basis of developmental sequence change. Coupled with studies that assess fitness implications, there is real potential to take a truly integrated approach to the study of developmental sequences using gastropods (Spicer and Rundle 2006).

LITERATURE CITED

Arthur, W. 2002. The emerging conceptual framework of evolutionary developmental biology. *Nature* **415**: 757-764.

Baldwin, E. 1935. The energy sources in ontogenesis. VIII The respiratory quotient of developing gastropod eggs. *Journal of Experimental Biology* **12**: 27-35.

Bielefeld U. and W. Becker. 1991. Embryonic development of the shell in *Biomphalaria glabrata* (Say). *International Journal of Developmental Biology* **35**: 121-131.

Bininda-Emonds, O. R. P., J. E. Jeffery, M. I. Coates, and M. K. Richardson. 2002. From Haeckel to event-pairing: The evolution of developmental sequences. *Theory in Biosciences* **121**: 297-320.

Bininda-Emonds, O. R. P., J. Jeffrey, and M. K. Richardson. 2004. Is sequence heterochrony an important evolutionary mechanism in mammals? *Journal of Mammalian Evolution* **10**: 335-359.

Blochmann, F. 1882. Über die Entwicklung der *Neritina fluviatilis* Mül. *Zeitschrift für wissenschlaftliche Zoologie* **36**: 125-174 [In German].

Collin, R. 2004. Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. *Evolution* **58**: 1488-1502.

Collin, R. and J. B. Wise. 1997. Morphology and development of *Odostomia columbiana* Dall & Bartsch (Pyramidellidae): Implications for the evolution of gastropod development. *Biological Bulletin* **192**: 243-252.

Conklin, E. G. 1897. The embryology of *Crepidula*, a contribution to the cell lineage and early development of some gastropods. *Journal of Morphology* **13**: 1-266.

Croll, R. P. and E. E. Voronezhskaya. 1996. Early elements in gastropod neurogenesis. *Developmental Biology* **173**: 344-347.

Cummin, R. 1972. Normentafel zur Organogenese von *Lymnaea stagnalis* (Gastropoda Pulmonata) mit besonderer Berücksichtigung der Mitteldarmdrüse. *Revue Suisse Zoologie* **79**: 709-774 [In German].

deBeer, G. 1958. *Embryos and Ancestors*, 3rd Edition. Clarendon Press, Oxford, U.K.

Delsman, H. C. 1914. Entwicklungsgeschichte von *Littorina obtusata*. *Tijdschrift der Nederlandsche Dierkundige Vereeniging* **14**: 383-498 [In German].

Freeman, G. and J. W. Lundelius. 1992. Evolutionary implications of the mode of D quadrant specification in coelomates with spiral cleavage. *Journal of Evolutionary Biology* **5**: 205-247.

Fretter, V. and A. Graham. 1962. *British Prosobranch Molluscs: Their Functional Anatomy and Ecology.* Ray Society, London.

Garstang, W. 1922. The theory of recapitulation. A critical restatement of the biogenetic law. *Journal Linnean Society, London* (Zoology) **35**: 81-101.

Gibson, G. D. 2003. Larval development and metamorphosis in *Pleurobranchaea maculata*, with a review of development in the Notaspidea (Opisthobranchia). *Biological Bulletin* **205**: 121-132.

Gould, S. J. 1977. *Ontogeny and Phylogeny*. Harvard University Press, Cambridge, Massachusetts.

Haeckel, E. H. P. A. 1866. *Generelle Morphologie der Organismen allgemeine Grundzüge der organischen Formen-Wissenschaft: mechanisch begründet durch die von Charles Darwin reformirte Descendenz-Theorie*. Vol. 1-2. G. Reimer, Berlin [In German].

Hejnol, A., M. Q. Martindale, and J. Q. Henry. 2007. High-resolution fate map of the snail *Crepidula fornicata*: The origins of ciliary bands, nervous system, and muscular elements. *Developmental Biology* **305**: 63-76.

Hinman, V. F., E. K. O'Brian, G. S. Richards, and B. M. Degnan. 2003. Expression of anterior *Hox* genes during larval development of the gastropod *Haliotis asinina*. *Evolution and Development* **5**: 508-521.

Hyman, L. H. 1951. *The Invertebrates: Platyhelminthes and Rhynchocoela, the Acoelomate Bilateria*, Vol. 2. McGraw-Hill, New York.

Jeffery, J. E., M. K. Richardson, M. I. Coates, and O. R. P. Bininda-Emonds. 2002a. Analyzing developmental sequences within a phylogenetic framework. *Systematic Biology* **51**: 478-491.

Jeffery, J. E., O. R. P. Bininda-Emonds, M. I. Coates, and M. K. Richardson. 2002b. Analyzing evolutionary patterns in vertebrate embryonic development. *Evolution and Development* **4**: 292-302.

Jeffery, J. E., O. R. P. Bininda-Emonds, M. I. Coates, and M. K. Richardson. 2005. A new technique for identifying sequence heterochrony. *Systematic Biology* **54**: 230-240.

Lartillot, N., O. Lespinet, M. Vervoort, and A. Adouette. 2002. Expression pattern of *Brachyury* in the mollusc *Patella vulgata* suggests a conserved role in the establishment of the AP axis in Bilateralia. *Development* **129**: 1411-1421.

Lindberg, D. R. and R. P. Guralnick. 2003. Phyletic patterns of early development in gastropod molluscs. *Evolution and Development* **5**: 494-507.

Mabee, P. M. and T. A. Trendler. 1996. Development of the cranium and paired fins in *Betta splendens* (Teleosti: Percomorpha): Intraspecific variation and interspecific comparisons. *Journal of Morphology* **227**: 249-287.

Nunn, C. L. and K. K. Smith. 1998. Statistical analysis of developmental sequences: The craniofacial region in marsupial and placental mammals. *American Naturalist* **152**: 82-101.

Page, L. R. 1994. The ancestral gastropod larval form is best approximated by hatching-stage opisthobranch larvae: Evidence from comparative developmental studies. *In*: W. H. Wilson Jr., S. A. Stricker, and G. L. Shinn, eds., *Reproduction and Development of Marine Invertebrates*. The Johns Hopkins University Press, Baltimore and London. Pp. 206-223.

Page, L. R. 1998. Sequential developmental programmes for retractor muscles of a caenogastropod: Reappraisal of evolutionary homologues. *Proceedings of the Royal Society* (B) **265**: 2243-2250.

Ponder, W. F. and D. R. Lindberg. 1997. Towards a phylogeny of gastropod molluscs: An analysis using morphological characters. *Zoological Journal of the Linnean Society, London* **119**: 83-265.

Raff, R. A. 1996. *The Shape of Life*. University of Chicago Press, Chicago.

Raff, R. A. 2000. Evo-devo: The evolution of a new discipline. *Nature Reviews Genetics* **1**: 74-79.

Raven, C. P. 1958. *Morphogenesis: The Analysis of Molluscan Development*. Pergamon Press, London.

Render, J. 1997. Cell fate maps in the *Ilyanassa obsoleta* embryo beyond the third division. *Developmental Biology* **189**: 301-310.

Richardson, M. K. 1995. Heterochrony and the phylotypic period. *Developmental Biology* **172**: 412-421.

Richardson, M. K. 1998. Haeckels's embryos continued. *Science* **281**: 1289.

Richardson, M. K. and J. E. Jeffery. 2002. Haeckel and modern biology. *Theory in Biosciences* **121**: 247-251.

Richardson, M. K. and G. Keuck. 2002. Haeckel's ABC of evolution and development. *Biological Reviews* **77**: 495-528.

Richardson, M. K., J. E. Jeffery, M. I. Coates, and O. R. P. Bininda-Emonds. 2001. Comparative methods in developmental biology. *Zoology* **104**: 278-283.

Schulmeister, S. and W. C. Wheeler. 2004. Comparative and phylogenetic analysis of developmental sequences. *Evolution and Development* **6**: 50-57.

Smirthwaite, J. J., S. D. Rundle, O. R. P. Bininda-Emonds, and J. I. Spicer. 2007. An integrative approach identifies developmental sequence heterochronies in freshwater basommatophoran snails. *Evolution and Development* **9**: 122-130.

Smith, K. K. 1996. Integration of craniofacial structures during development in mammals. *American Zoologist* **36**: 70-79.

Smith, K. K. 1997. Comparative patterns of craniofacial development in eutherian and metatherian mammals. *Evolution* **51**: 1663-1678.

Smith, K. K. 2001. Heterochrony revisited: The evolution of developmental sequences. *Biological Journal Linnean Society, London* **73**: 169-186.

Smith, K. K. 2003. Time's arrow: Heterochrony and the evolution of development. *International Journal of Developmental Biology* **47**: 613-621.

Spicer, J. I. and W. W. Burggren. 2003. Development of physiological regulatory systems: Altering the timing of crucial events. *Zoology* **106**: 91-99.

Spicer, J. I. and S. D. Rundle. 2006. Out of place and out of time: Towards a more integrative approach to heterochrony. *Animal Biology* **57**: 487-502.

Spicer, J. I. and S. D. Rundle. 2007. Plasticity in the timing of physiological development: Physiological heterokairy—what is it, how frequent is it, and does it matter? *Comparative Biochemistry and Physiology* (A) **148**: 712-719.

Taylor, H. H. 1977. The ionic and water relations of embryos of *Lymnaea stagnalis*, a freshwater pulmonate mollusc. *Journal of Experimental Biology* **69**: 143-172.

van den Biggelar, J. A. M. 1993. Cleavage pattern in embryos of *Haliotis tuberculata* (Archaeogastropoda) and gastropod phylogeny. *Journal of Morphology* **216**: 121-139.

van den Biggelar, J. A. M. and G. Haszprunar. 1996. Cleavage patterns and mesentoblast formation in the Gastropoda: An evolutionary perspective. *Evolution* **50**: 1520-1540.

Wanninger, A., D. Koop, S. Moshel-Lynch, and B. M. Degnan. 2008. Molluscan evolutionary development. *In*: W. F. Ponder and D. R. Lindberg, eds., *Phylogeny and Evolution of the Mollusca*. University of California Press, Berkeley and Los Angeles. Pp. 427-446.

Yamanaka, M., D. Hatakeyama, H. Sadamoto, T. Kimura, and E. Ito. 2000. Development of key neurons for learning stimulates learning ability in *Lymnaea stagnalis*. *Neuroscience Letters* **278**: 113-116.

Submitted: 9 November 2008; **accepted:** 9 February 2009;
final revisions received: 16 April 2009